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SOME PHENOMENA INVOLVED IN THE LIFE HISTORY OF SPIROCHAETA SUIS.*†

STUDIES ON HOG CHOLERA.

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The recognition of *Spirochaeta suis*[1] in certain lesions found in cases of hog cholera, and the results attending experimental cultivation and animal inoculation have afforded many interesting observations relative to the life history of the organism. These studies have not yet progressed to such a point that definite conclusions can be drawn as to the cycle through which this organism may pass. However, its morphological variations under different conditions appear to correspond in many respects with those of other spirochetes which have been studied and reported upon by many investigators.[2]

A future publication, which we hope will contribute toward the interpretation of the life cycle of spirochetes in general, will contain more detailed results of our observations of *Spirochaeta suis*, and its morphological and biological variations. This preliminary report is submitted in order that the results of certain filtration experiments may be placed on record.

Dujardin-Beaumetz[3] in discussing *Die Peripneumonie der Rinder* refers to certain interesting filtration experiments as follows:

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† Owing to the provisional use of the name "Spirochaeta suis" by Bosanquet (*Spirochetes*, Saunders, 1911) and Neveu-Lemaire (*Parasitologie des Animaux Domestiques*, Paris, 1912) in referring to the findings of Dodd (*Jour. Comp. Path.*, 1906, 19, p. 216) and *Cleland* (*Parasitology*, 1908, 1, p. 218), it will be necessary to designate this organism by another name. This will be done in future publications.

[1] King and Baeslack, *Jour. Infect. Dis.*, 1913, 12, p. 307; King, Baeslack, and Hoffmann, *Jour. Infect. Dis.*, 1913, 12, p. 365; King and Hoffmann, *Jour. Infect. Dis.*, 1913, 13, p. 463.

[2] Gleitsmann, *Centralbl. f. Bakteriol.*, I, *Orig.*, 1913, 68, p. 31; Mühlens, *Handbuch d. Path. Protozoen* Prowazek, Leipzig, 1912, p. 361; Ross, *Brit. Med. Jour.*, 1912, p. 1651; McDonagh, *Lancet*, *Proc. Roy. Soc.*, 1913, 6, p. 86; Dutton, *Jour. Trop. Med.*, 1907, 10, p. 385; Mayer, *Arch. f. Schiffs.- u. Tropenhyg.*, 1908, 12, *Beihefte* 1, p. 1; Mackinnon, *Parasitology*, 1909, 2, p. 267; Bosanquet, *Spirochetes*, Saunders, 1911; Marchoux and Couvy, *Ann. de l'Inst. Past.*, 1913, 27, p. 450; Meirowski, *München. med. Wchnschr.*, 1913, 60, pp. 1870 and 2042. Fry, Ranken, and Plimmer, *Jour. Royal Army Med. Corps*, 1913, 21, p. 137; Balfour, *Centralbl. f. Bakteriol.*, I, *Orig.*, 1913, 70, p. 182; O'Farrell and Balfour, *Centralbl. f. Bakteriol.*, I, *Ref.*, 1913, 59, p. 292; Noguchi, *Jour. Exper. Med.*, 1912, 16, p. 199.

[3] *Handbuch d. Path. Mikroorg.*, 1913, 8, p. 943.

"ISOLATION OF BACTERIA BY FILTRATION."

"In investigations of pleuropneumonia, various writers have often filtered the pleuropneumonia exudate through 'Gips' and porcelain filters and observed that such filtrate is incapable of causing either infection or immunity in the animals. From this fact it was learned that the pleuropneumonia was not caused by a soluble virus, but by a living organism. But when Löffler had proved that it was possible to infect animals with lymph obtained from aphthous pustules, diluted with water in proportion 1:50 and then filtered, and that there were therefore bacteria which were so infinitely small that they would pass through the filter, the investigations on pleuropneumonia were again taken up, along the lines where positive results were obtained by means of the dilution method. The method of dilution, as well as the quality of the filter, is of the greatest importance for the success of the experiment. If pleuropneumonia exudate or a culture in bouillon, which contains more that 15 per cent of serum, is used for filtration without being previously diluted, failure is usually the result, since the pores of the filter become clogged with the albumen. The choice of candle is not without significance as the different filters possess different degrees of porosity. . . . . The passage of this organism through certain filtering walls is of the greatest interest from the standpoint of bacteriologic technic. The filter, which until now has been regarded as a perfect process for the sterilization of warmed fluid media, can also be of great service in the isolation of very small bacteria, by retaining the other larger microbes in their walls. It might also be possible to isolate and compare the filtrable viruses serially, using for this a set of suitably chosen filters of decreasing porosity.

"If the possibility is also offered of obtaining the pleuropneumonia virus in pure state, it is always wise to inoculate cultures in serum bouillon. If the presence of foreign bacteria in the inoculation material is feared, a preliminary culture on serum agar can be made; this, however, is not advisable. Pleuropneumonia develops very slowly on solid media, and the colonies are not visible until after 3 days' standing at 37°. During this time the other more rapidly developing bacteria, such as the pleuropneumonia bacillus of Arloing, overgrow the surface of the agar, and render isolation of the pleuropneumonia organism impossible. By employment of the filtration method this obstacle is overcome. The fluid can be collected without any special precautions. It can be taken even in the stage of decomposition and still be capable, by filtration, of isolating the organism in pure state. The process is as follows: The lung lymph is diluted in proportion of $\frac{1}{2}$:100 with peptone bouillon containing no serum. The bouillon must be filtered through a porcelain filter, not for purpose of sterilization, but to eliminate all solid particles which are suspended in the fluid and could hinder filtration. The lymph diluted in the above manner is then filtered through Berkefeld or Chamberland filters L_2. It is recommended that the bouillon be first warmed at 37–39°, for it is known that warm fluids can pass through porous strata better, and the passage of the virus is thereby guaranteed. For the certainty of the growth of the specific cause, serum must be added to the filtrate so obtained, in the proportion of 10 parts serum to 100 parts bouillon. To prevent accidental contamination, it is well to filter the serum through the same filter as is used for the filtration of the bouillon. The filtrate, mixed with serum, is placed in the incubator, and after 3–4 days an opalescence is observed in the cultures. If such an opalescence also appears in those tubes which were further inoculated with material from these cultures, and if no such change can be observed in the control tubes which were inoculated at the same time, the growth of the pleuropneumonia virus is proved, and the costly animal experiment is unnecessary. It is sufficient if only a trace of the opalizing fluid is spread on the serum agar. Very fine colonies, at first transparent, later wartlike, appear on this medium in so characteristic a form as to banish any doubt as to their identity.

"This method can be used not only for the purpose of isolation, but also for veterinary diagnosis in the investigation of lung material obtained from cattle which have succumbed to suspected pneumonia."

In a former publication[1] data were presented showing that the Berkefeld filtrates of cultures containing *Spirochaeta suis* were capable of producing hog cholera in susceptible hogs. In the meantime successful attempts have been made to develop cultures of the spirochete from the Berkefeld filtrates. This work, of which brief notes are given, was undertaken chiefly for the purpose of securing data bearing on three important points: (1) to determine the correlation between the "filtrable virus," or "ultramicroscopic organism" of hog cholera, and *Spirochaeta suis;* (2) to secure pure cultures of the spirochete; and (3) to determine the importance of the granules assumed to be related to *Spirochaeta suis.*

September 23, 1913: A small portion of necrotic tissue containing numerous *Spirochaetae suis*, from ear of Hog 653, was shaken in sterile water and filtered through Berkefeld filter N, pressure less than one atmosphere, time of filtration about 20 mins., and volume of filtrate about 10 c.c. Cultures from the filtrate were made in broth, grown aerobically, and found to be sterile.

Special media, inoculated with the filtrate and incubated under anaerobic conditions, were placed in a desiccator, *in vacuo*, with pyrogallic acid. The media consisted of (1) Hata medium,[2] with hog blood corpuscles, and (2) Hata medium with rabbit kidney tissue and 0.5 per cent phenol.

DARK-FIELD EXAMINATIONS.

October 18, 1913: One culture of each medium showed many granules but no spirochetes.

October 31, 1913: One culture of each showed slight contamination with a bacillus, no spirochetes, no granules noted.

November 19, 1913: Two cultures of each showed some contamination, bacillus.

December 9, 1913: Three cultures of each, same as on November 19.

December 15, 1913: Cultures examined, contaminating bacillus found in all. *Spirochaeta suis* found in 2 cultures of Hata medium, rabbit kidney, and 3 cultures of Hata medium, hog corpuscles.

Spirochetes were not numerous, but were found with little difficulty, and some were actively motile.

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[1] *Jour. Infect. Dis.*, 1913, 13, p. 484.

[2] Hata, paper read at Seventeenth International Congress of Medical Sciences, London, August, 1913.

December 17, 1913: One of contaminated cultures of *Spirochaeta suis*, examined under date of December 15, 1913, shaken in sterile water and filtered through Berkefeld. The filtrate was inoculated into plain broth, agar slant, and deep glucose agar, and after incubation under both aerobic and anaerobic conditions, no growth resulted.

December 19, 1913: Special media—(1) Hata medium with hog kidney, (2) hog serum medium, with and without hog kidney, and (3) pyrogallic serum medium (Proca, Danila, and Stroe[1]) with hog kidney—were inoculated with Berkefeld filtrate of original culture, No. 653, *Spirochaeta suis*.

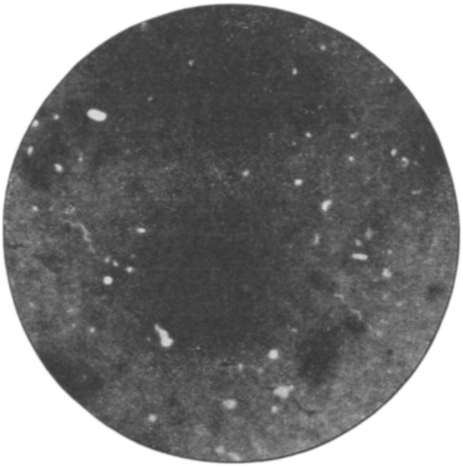

FIG. 1.—Photomicrograph of *Spirochaeta suis* in culture from Berkefeld filtrate of suspension of material, ear lesion, Hog 653. ×1200. (India ink preparation.)

DARK-FIELD EXAMINATIONS.

December 21, 1913: One culture of each medium examined. Contaminating organisms (bacilli and cocci) found. No spirochetes present.

December 24, 1913: Two cultures of each medium examined, same result as on December 21, 1913.

December 29, 1913: Three cultures of each medium examined, same result as on December 24, 1913. As all cultures in this lot appeared to be contaminated, they were discarded. From the check culture tests made, it was evident that contamination with bacilli and cocci was present in the special culture media. Repeated results on other occasions confirm the conclusion that contamination of these special media occurs as the result of adding the kidney tissue and hog corpuscles to the medium after it is prepared.

January 5, 1914: Culture 653, containing *Spirochaeta suis*, first generation, described under first section of these notes, was shaken in sterile water and passed through Berkefeld filter as above. Filtrate was found to contain no organisms capable of developing on broth, agar, or deep glucose agar culture media, when incubated aerobically and anaerobically for several days.

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[1] *Compt. rend. Soc. de biol.*, 1912, 72, p. 895.

New lot of special deep media, some containing no kidney tissue or blood clot, was inoculated with filtrate, sterile except for granules presumably related to *Spirochaeta suis*.

DARK-FIELD EXAMINATIONS.

January 9, 1914: Two cultures examined, nothing detected except numerous granules in one culture.

January 12, 1914: Several cultures examined. *Spirochaeta suis* found in two cultures. Not actively motile, small, consisting of about 2–3 convolutions (old cultures show spirochetes of 4–10 convolutions, average number, perhaps 6).

January 15, 1914: Third culture found to contain spirochetes, morphology similar to those noted on January 12, 1913.

From the above short series of experiments no definite conclusions should be drawn. One must recognize the fact that filtration experiments are difficult to control and many erroneous results have been recorded in the literature from time to time.

The results of the experiments outlined in this paper have been controlled by logical methods, and are suggestive of the theory that *Spirochaeta suis*, at some period in its life cycle, is capable of passing through bacteria-proof filters.